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THE WILT DISEASE OF SAFFLOWER

THE ROYAL CANADIAN INSTITUTE

BY

S. D. JOSHI, B.Sc

Research Assistant, Plant Pathological Section

Department of Agriculture, United Provinces

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THE WILT DISEASE OF SAFFLOWER.

BY

S. D. JOSHI, B.Sc.,

Research Assistant, Plant Pathological Section,

Department of Agriculture, United Provinces.

[Received for publication on 15th October, 1923.]

THE safflower (*Carthamus tinctorius*) is grown in many parts of India. In the United Provinces, it is grown as a winter crop largely in the Bulandshahr and Meerut Districts and in small quantities in the eastern districts either as a mixed crop or along the borders.

The increasing economic importance of the crop in India has stimulated its extensive study at all quarters. Howard and Remington[1] have shown its economic value on account of the oil and the dye it yields.

The wilt disease of safflower was for the first time observed at Pusa in the winter of 1920 when the crop was badly affected at the flowering time. Later on it was also found at Sankni in the district of Bulandshahr. The cultivators hold that the disease is of more common occurrence in years of abundant rainfall. This is but to be expected when we consider the life-habits of the causal organism.

The extent of the damage varies according to the character of the season at the time of infection.

The number of plants affected in the fields at Pusa was as high as 30 per cent. in some of the varieties, practically all of which were involved. The disease was brought to notice only when a large number of mature plants began to show sudden wilting; therefore it was not possible to make observations as to how the disease progressed in the field.

Sporadic cases of wilt were known to occur in the safflower crop every year, but no serious notice was taken of it. Although the plots which were badly

[1] Howard, A., and Remington, J. S. *Pusa Agri. Res. Inst. Bull.* 124.

affected were not under safflower in the preceding season, the fungus might have been present in the crop which was grown there the year before. The fungus also attacks varieties of other crops and weeds and through them it can be carried from year to year; at the same time the infective material in the form of sclerotia is increased considerably which causes the disease to spread over increasingly wider areas.

It is difficult to formulate the exact conditions which led to a severe attack at Pusa in 1919-20. Ploughing in of the stubbles of the previous crop might have increased the infective material considerably. The favourable temperatures of between 50°–60°F. in January and February combined with high humidity accompanying a rainfall of 1·7″ in February and 1·11″ in March favoured the growth of the fungus and resulted in the severe damage observed. The earliest cases of wilt were noticed in a few plants towards the end of January.

Symptoms.

The wilted plants have dense white growth of mycelium at their bases. Large black sclerotia are produced just below the soil level, on the surface of the crown and roots. These are very loosely attached so that the plants have to be carefully pulled up to get the sclerotia in position. The sclerotia are also produced inside the stem (Pl. I, fig. 5). In the diseased crop at Pusa an apparently healthy grown up plant showed the next day a little yellowing of the leaves and very quickly the whole plant dried up. The cortical tissue in the lower part of the stem came off into shreds. A characteristic feature of the diseased plant at an advanced stage was the ease with which the flower-heads broke off from their stalks, leaving behind an outer involucre of bracts. In a healthy plant, on the other hand, the flower-heads are firmly attached to the stalks and cannot easily be separated. This condition was due to the development of a large black pear-shaped sclerotium in the thalamus of the flower (Pl. III, fig. 3) and the thalamus changing into a powdery mass.

The attacked plants in some cases resisted wilting and produced apparently normal fruits. These fruits, however, at maturity, were found to contain either no seeds or only defective seeds.

Cultural characters.

The mycelium is of the typical *Rhizoctonia* form showing the usual constriction and septation in branching (Pl. I, fig. 1). The cells in young culture show a great variation in their size. They are from 115μ to 190μ in length by 16μ to 21μ in breadth. The first septum is usually formed at a distance of 20μ to 47μ from the parent hypha.

The sclerotia are very irregular in shape (Pl. I, fig. 5), varying from roundish to elongate. They are thick solid bodies about 2-12 mm. long. Those formed at the bases of flower-heads have in majority of cases a curious pear-shaped appearance, after the shape of the conical thalamus (Pl. III, fig. 1). The sclerotia begin as white hard spots in the mycelium; the outer surfaces afterwards turn black. Drops of a clear shining liquid are very prominent on the developing sclerotia and can be seen even after they have blackened. The sclerotia are formed on the surface of the mycelium from which they can be easily detached. Usually they have a covering of a semi-persistent thin mycelial membrane. Their outer surface is somewhat rough. The interior is formed by not very compactly interwoven hyphæ and thus when placed in water they float for a time. The outer two or three layers of the sclerotia appear brownish black while the interior is white (Pl. I, fig. 6). Often the recently formed sclerotia, in artificial cultures, show a very faint tinge of pink inside.

Peculiar tufty branchings (Pl. I, figs. 3, 4) are frequently formed in cultures of the fungus especially in glucose agar medium. These are seen at the edges of the slant cultures where the mycelium comes in contact with the surface of the glass. These branches are comparable to the branchings of *Botrytis* and *Sclerotinia* described by Smith[1] and may similarly be called the organs of attachment or appressoria.

Perfect apothecial stage has not been obtained so far. The sclerotia placed in moist sand and sawdust did not produce any apothecia. In a few cases, however, where they were sown on ordinary moist earth in a large petri dish the sclerotia gave out brownish outgrowths, about 4-10 mm. long, which developed distinct apothecial cups at their tips (Pl. I, figs. 2, 7), but inspite of every care they shrivelled up before any asci were produced. In cultures on corn, oat, wheat and bean-meal agar, there was sometimes development, from the surface of the sclerotia, of slender stalks either singly or in large numbers measuring as much as 2 cm. in length (Pl. II, fig. 4; Pl. III, fig. 2). They remained sterile till they dried up. Light appears to have little effect upon the development of these stalks.

No apothecial outgrowths could be found even with frequent and careful search in the fields, throughout the year.

A distinct spore form occurs in cultures 9 or 10 days old. The fertile hyphæ bear conidiophores as simple branches which may repeatedly branch to form a cluster of sterigmata (Pl. II, figs. 1, 2). These bear conidia at their

[1] Smith, R. E. *Botrytis* and *Sclerotinia*; their relation to certain plant diseases and to each other. *Bot. Gaz.*, p. 29, 1900.

tips in chains. The conidia are from 3-3·5μ in diameter, hyaline, with usually a highly refractive granule inside. Such spore form is always abundantly produced in bean, corn, wheat, oatmeal media, while the glucose agar medium although showing luxuriant growth of mycelium does not seem very suitable for it. The fungus does not grow well on safflower seed medium. These conidia could not be made to germinate. They failed to germinate in water, 1 per cent. glycerine, 1 per cent. cane sugar, turnip juice, horsedung solution, cowdung solution, and safflower leaf juice either at the ordinary winter temperatures of 20°–22°C. or at the higher temperature of 37°C. Efforts on glucose agar medium were equally unsuccessful.

Pathogenicity.

Germinating seedlings inoculated with bits of hyphæ from a pure culture rotted very quickly, forming copious mycelial growth on the surface which in time produced sclerotia. Young plants inoculated with the fungus at the soil level quickly succumbed and the fungus spread through the soil to the neighbouring healthy plants and soon killed them. Large plants with hard tissues are not killed immediately. The spread of the mycelium within the tissues of the host depends upon the moisture condition of the soil and atmosphere. That from the infected spots was completely checked when the plant was placed in dry open air. In all these cases the fungus could easily be recovered from infected plants in pure culture.

The mycelium grows in the tender tissues of the leaf and stem (Pl. II, fig. 3) and causes disorganization of the cells, changing them into soft pulpy mass. It penetrates the inner tissues of the stem and also grows in the pith where it forms large black sclerotia. The young plants so attacked quickly die while the maturer plants withstand wilting for some time.

It can be gathered from the above that if the conditions are favourable for the growth of the fungus when the plants are very young, a wholesale destruction of the crop may not be an improbability. Nevertheless, depending upon the time of infection and the rapidity of the spread of the disease in the field we meet with wilting of plants at different stages of their growth.

Hosts of the fungus.

A few other plants in the field besides safflower were also found infected with this fungus. Inoculations were, therefore, made on some of the more important winter crops and weeds. Wheat, oat, gram, mustard, pea, and potato, all took infection readily. Of the farm weeds the most common *Chenopodium album* (vernacular *bathua*) and *Asphodelus tenuifolia* (vernacular

pyazi) were very highly susceptible, while *Argemone* was less so and *Melilotus indica* did not take the infection at all.

Longevity of the sclerotia.

The sclerotia of the fungus do not seem to live very long. Those produced in the culture tubes between October and December 1920 showed very little germination in November 1921, and practically all in the tubes rotted away by October 1922. Only 14 per cent. of the sclerotia were found germinating after one year. Sclerotia which were gathered from the host plants in March 1920 grew in November 1920, but no germination was obtained in November 1921 or in 1922 and 1923 although they were still apparently sound.

Systematic.

The morphological characters of the safflower wilt fungus are exactly alike those of the large sclerotia type of *Sclerotinia Libertiana* Fuckl. described by Smith.[1] The sclerotia of this fungus although do not fruit easily, in rare cases have been found to produce distinct apothecial cups. A wilt of sunflower very closely resembling in effects that of safflower has been described by Bisby[2] in Manitoba and by Morris and Swingle[3] in Montana. The latter workers also found the sclerotia of their fungus which they identified as *S. Libertiana* fruiting with difficulty. A stalk disease of potato which is also similar to this wilt of safflower has been described by Pethybridge.[4] This latter he holds to be due to *Sclerotinia sclerotiorum* which according to him was " then known as *S. Libertiana* Fuckl., but known at an earlier date as *Peziza sclerotiorum* Lib., and now in accordance with the very widely accepted international rules of botanical nomenclature more correctly called *Sclerotinia sclerotiorum* Mass." This fungus, therefore, which causes the safflower wilt may be called *Sclerotinia sclerotiorum* Mass.

The spore form of the fungus described before may, however, give rise to certain doubt regarding its probable relationship with *Botrytis*. Brierley[5] in his recent studies on *Botrytis cinerea* noticed a microconidial stage associated with the normal type of conidia. This microconidial stage may be comparable

[1] Smith, R. E. *Loc. cit.*

[2] Bisby, G. R. *Sclerotinia* disease of sunflower in Manitoba. Abstracts in *Phytopathology*, XI, No. 1.

[3] Morris, H. E., and Swingle, D. B. An important new disease of cultivated sunflower. Abstracts in *Phytopathology*, XI, No. 1.

[4] Pethybridge, G. H. Investigations on potato diseases. *Jour. Dept. Agri. and Tech. Inst. Ireland*, XVI, No. 4.

[5] Brierley. The microconidia of *Botrytis cinerea*. *Kew Roy. Bot. Gardens Misc. Bull.* 4, 1918.

to the conidial stage of this fungus. From his own investigations and those of many others Brierley is of opinion that certain factors can drastically alter the normal life-cycle of *B. cinerea* and that any spore stage may be reversed or eliminated. But unlike Brierley as in his investigations, the writer has neither been able to get the conidia to germinate nor could he find out the circumstances under which a normal *Botrytis* stage, if any, may be formed in the cultures of safflower wilt fungus.

Some species of *Sclerotinia* described by De Bary[1] and Gilbert and Bennett[2] are known to produce similar spores either on the apothecia by the germination of ascospores or on the vegetative mycelium, which also did not germinate. As mentioned before, the conidia of this fungus cannot be germinated and can be regarded as functionless. It is indicated, therefore, that a *Botrytis* stage is absent from the life-cycle of this fungus.

Pethybridge[3] has given the authority of all workers to show that *Botrytis* stage is absent in *S. sclerotiorum* and that there is no connection between the two.

A distinguishing character of *S. sclerotiorum*, according to Tubeuf and Smith,[4] is the conical funnel-shaped depression in the hymenial disc. This depression which is clearly seen in this fungus (Plate I, fig. 7). Hence it is concluded that the fungus under study is *Sclerotinia sclerotiorum* Mass.

A comparison of *Rhizoctonia Napi* West. of Shaw and Ajrekar[5] with the safflower organism suggests their complete identity. Their morphological and anatomical characters are very much alike. As in safflower fungus, the faintly pink tinge of the interior of the sclerotia has been noticed in their sclerotia as well, which they have described as flesh-coloured (although in one place they describe it as white in the interior). The spore forms are similar. The range of host plants and the symptoms are almost the same. There is, therefore, every reason to believe that the *Rhizoctonia Napi* West. of these authors is really *Sclerotinia sclerotiorum* Mass.

A comparison of the description of these authors has led Pethybridge[6] also to suggest that it is clearly a species of *Sclerotinia*.

Sclerotinia sclerotiorum which is then of considerable economic importance in India may be regarded as a new addition to the few species of *Sclerotinia* so far recorded in this country.

[1] De Bary. *Comparative morphology and biology of fungi, mycetozoa and bacteria.*

[2] Gilbert, A. H., and Bennett, C. W. *Sclerotinia Trifoliorum*, the cause of stem rot of clover and alfalfa. *Phytopathology*, VII, No. 6.

[3] Pethybridge, G. H. *Loc. cit.*

[4] Tubeuf and Smith. *Diseases of plants induced by cryptogamic parasites*, p. 265.

[5] Shaw, F. J. F., and Ajrekar, S. L. The genus *Rhizoctonia* in India. *Mem. Dept. Agri. India, Bct. Ser.*, VII, No. 4.

[6] Pethybridge, G. H. *Loc cit.*

Control measures.

The temperatures which the sclerotia are able to withstand under diverse conditions are very difficult to determine with accuracy. The results of the immersion into water for 5 minutes are given in the table below.

Number of sclerotia	Temperature at which immersed	Number germinating	Remarks
	°C.		
10	98·8	nil	
10	80·0	..	
10	70·0	..	
10	60·0	..	
10	50·0	..	
10	40·0	9	The controls germinated in each case.
10	45·0	9	
25	46·0	18	
25	47·0	3	
25	48·0	1	
25	50·0	nil	
25	51·0	..	

The sclerotia were treated with corrosive sublimate solution, 1 in 1000, for about 15 minutes and washed with sterile water before immersion. They were afterwards planted in agar slants. The controls germinated in each case.

It will be seen from the above table that a temperature of between 48° – 50°C. approaches the thermal death point of the fungus. It is not, however, likely that the sclerotia will be killed by the summer soil temperatures which may not ordinarily be more than 40°C. at a few inches below the surface. Neither does there appear any chance of starving out the fungus with such a large number of host plants by a practice of long rotation. Any treatment of the soil by sterilization also is out of question from the point of view of expenses.

The fungus does not survive in the seed obtained from the diseased crop as such seed gave a clean crop in the following season.

Taubenhaus[1] found that infection with *Sclerotium Rolfsii* was not possible if the mycelium or sclerotia were buried more than 5" deep and successful inoculation was obtained only when the fungus was not covered more than ½"–1" deep. The writer's experience has been in the case of potato *Rhizoctonia* (identified by Shaw and Ajrekar as *Rhizoctonia destruens*) that the growth of

[1] Taubenhaus, J. J. Recent studies on *Sclerotium Rolfsii* Sacc. *Jour. Agri. Res.*, XVIII, 1919.

mycelium and the infection of tubers could be met with at about 5″ or 6″ below the surface provided the moisture is present and the temperature is suitable.

Smith[1] observed that sterilizing 3″ of the surface soil checked the disease due to *S. Libertiana* even in the most infected beds. It can be inferred from the above statement that either the sclerotia do not happen to pass down deeper than 3″ or that they do not germinate below that depth. A reasonable explanation seems to be that those sclerotia that remained in the upper 3″ might have been killed by sterilization while those below that depth failed to germinate. Following this argument as well as from the writer's experience it appears that burying the sclerotia very deep might considerably reduce infection.

The most practicable directions of control, therefore, are, firstly, the careful collection and destruction of infected plant material and, secondly, a very deep ploughing to secure the burial of any remaining sclerotia to a depth of more than 6″. Careful and clean weeding can prevent the rapid spread of the disease in the field.

Summary.

1. Low temperature and abundant moisture are very favourable to the growth of the fungus causing safflower wilt. The fungus grows on many different media.

2. The sclerotia do not seem to live longer than a little over one year.

3. The causal organism is *Sclerotinia sclerotiorum* Mass. It is identical with *Rhizoctonia Napi* West. of Shaw and Ajrekar, which should correctly be identified as such.

4. The sclerotia are killed by immersion in water at 50°C. for five minutes.

5. The practicable control measures are :—

(*a*) Collection and destruction of infective material.

(*b*) Deep ploughing.

(*c*) Clean weeding.

The writer is thankful to Dr. E. J. Butler, Director, Imperial Bureau of Mycology, London, for suggestion of the subject of investigation while working at Pusa, and desires to acknowledge his indebtedness to Mr. P. K. Dey, Plant Pathologist to Government, United Provinces, Cawnpore, for ever ready help and criticisms.

[1] Smith, R. E. *Loc. cit.*

SCLEROTINIA SCLEROTIORUM

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EXPLANATION OF PLATE I.

Fig. 1. Mycelium of *Sclerotinia sclerotiorum* Mass, from safflower. (× 553)
" 2. A sclerotium showing apothecial outgrowths. (× 2)
Figs. 3, 4. Forms of organs of attachment. (× 535)
Fig. 5. Stem of safflower showing sclerotia in the pith. (natural size)
" 6. Section of sclerotium. (× 480)
" 7. Section of an apothecial cup. (× 103)

[illegible] a little over [illegible]

[illegible] *Sclerotinia sclerotiorum* Mass. It is identical with *Rhizoctonia Napi* West. of Shaw and Ajrekar, which should correctly be identified as such.

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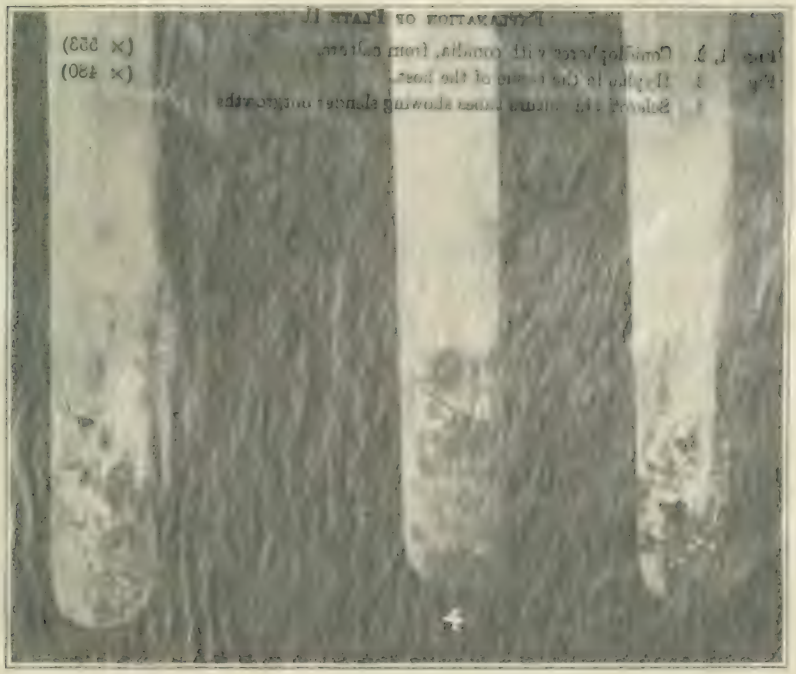
[illegible] Smith [illegible]

SCLEROTINIA SCLEROTIORUM



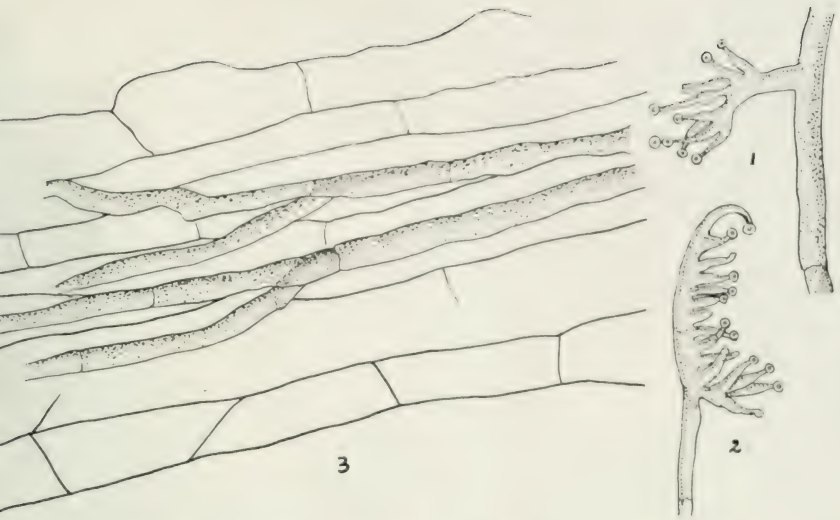
SCLEROTINIA SCLEROTIORUM.

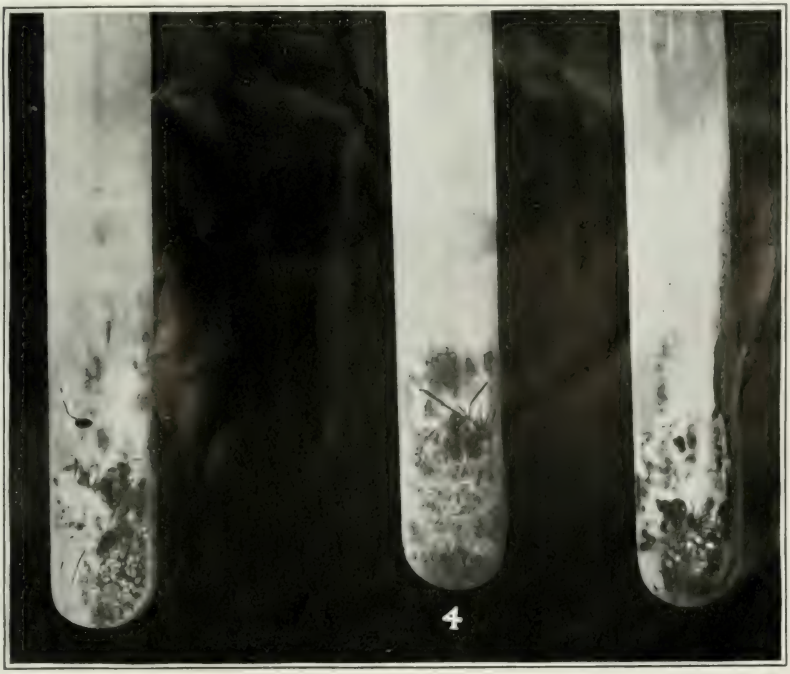
Explanation of Plate II.

Figs. 1, 2. Conidiophores with conidia, from culture. (× 553)

Fig 3. Hyphæ in the tissue of the host. (× 480)

„ 4. Sclerotia in culture tubes showing slender outgrowths.





SCLEROTINIA SCLEROTIORUM.

Explanation of Plate III

Fig. 1. Pear-shaped sclerotia from the flower of saffower.

" 2. Sclerotia showing the development of numerous slender stalks.

" 3. Flower-head detached from the thalamus showing the sclerotium inside. (½ natural size.)

Explanation of Plate III.

Fig. 1. Pear-shaped sclerotia from the flower of safflower.

,, 2. Sclerotia showing the development of numerous slender stalks.

,, 3. Flower-head detached from the thalamus showing the sclerotium inside. ($\frac{1}{2}$ natural size.)

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